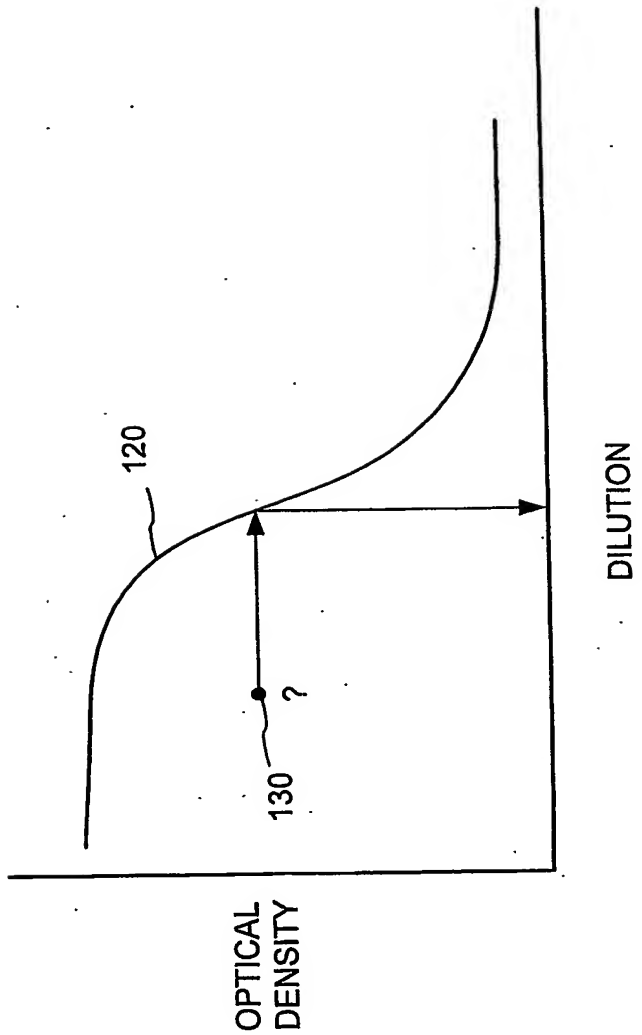


FIG. 1

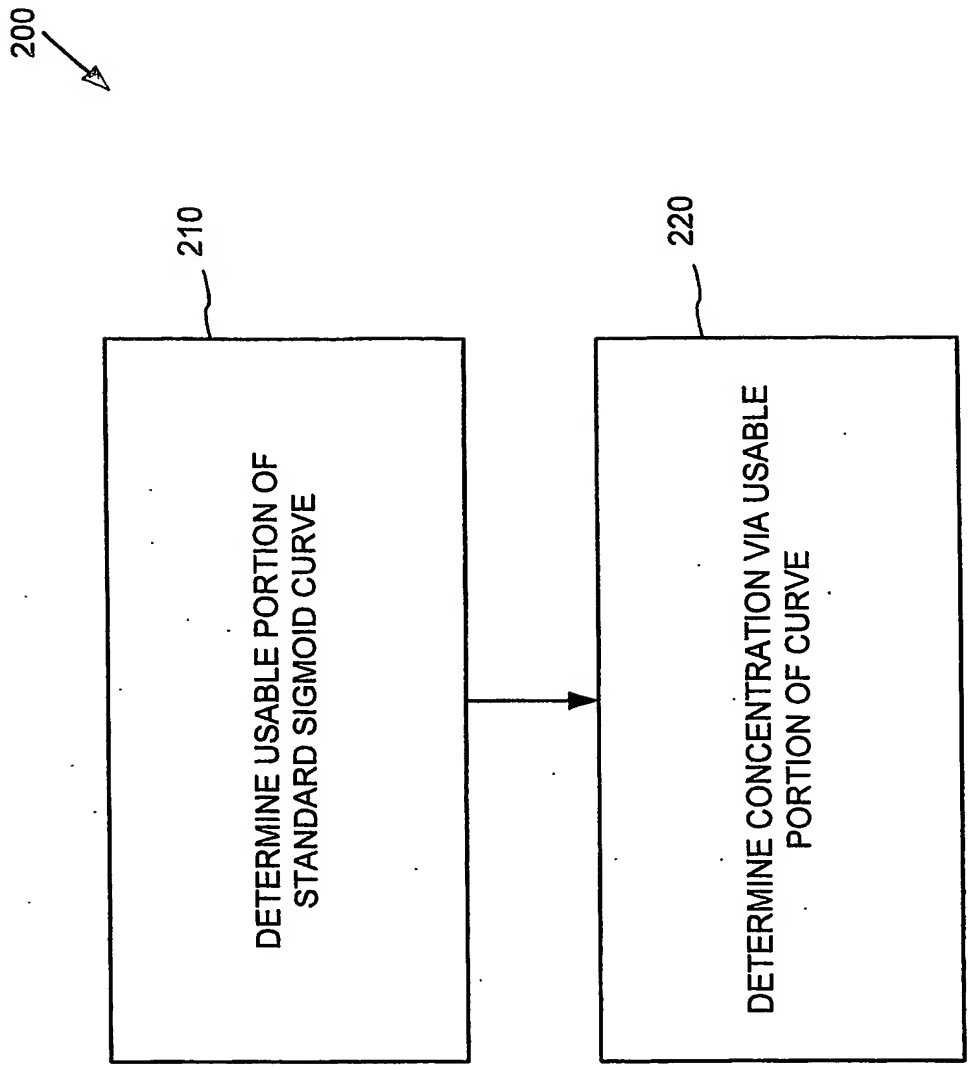

200
210
DETERMINE USABLE PORTION OF STANDARD SIGMOID CURVE
220
DETERMINE CONCENTRATION VIA USABLE PORTION OF CURVE

FIG. 2

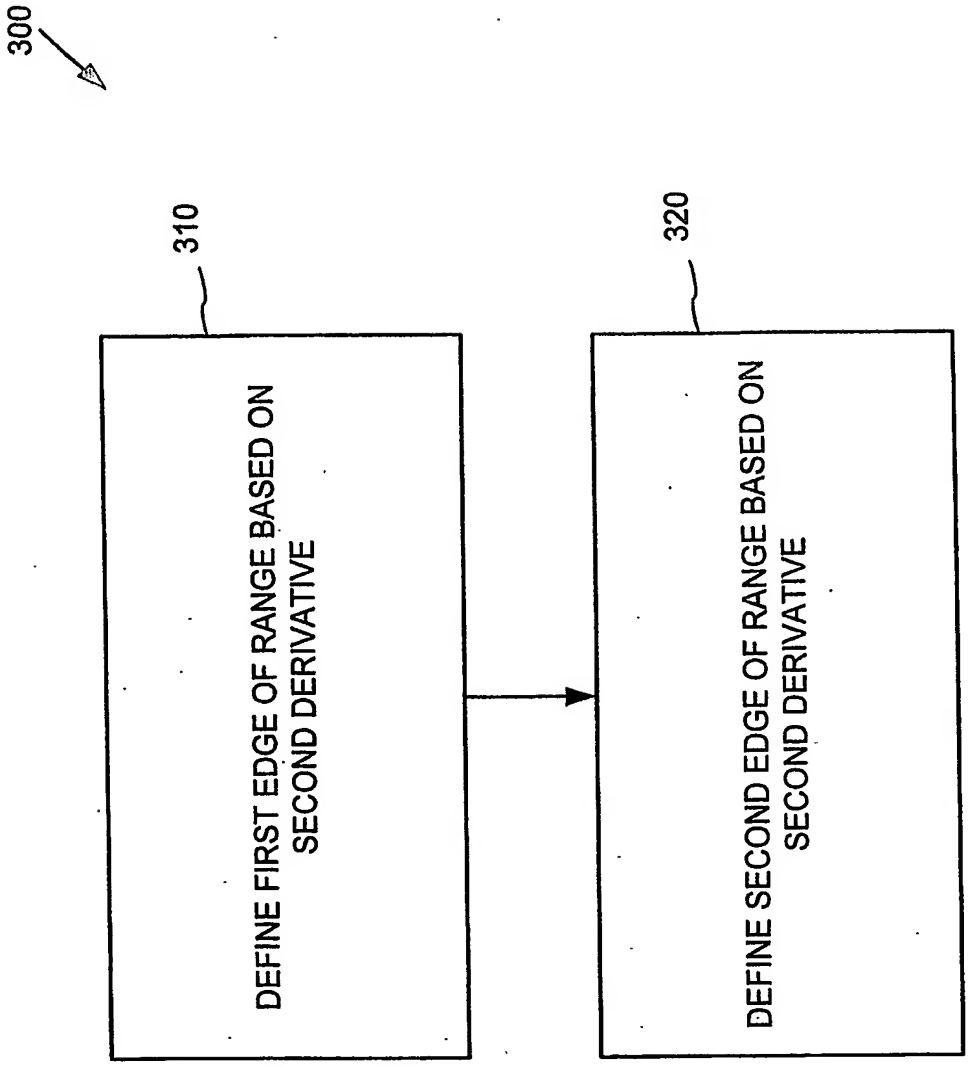

300
310
DEFINE FIRST EDGE OF RANGE BASED ON SECOND DERIVATIVE
320
DEFINE SECOND EDGE OF RANGE BASED ON SECOND DERIVATIVE

FIG. 3

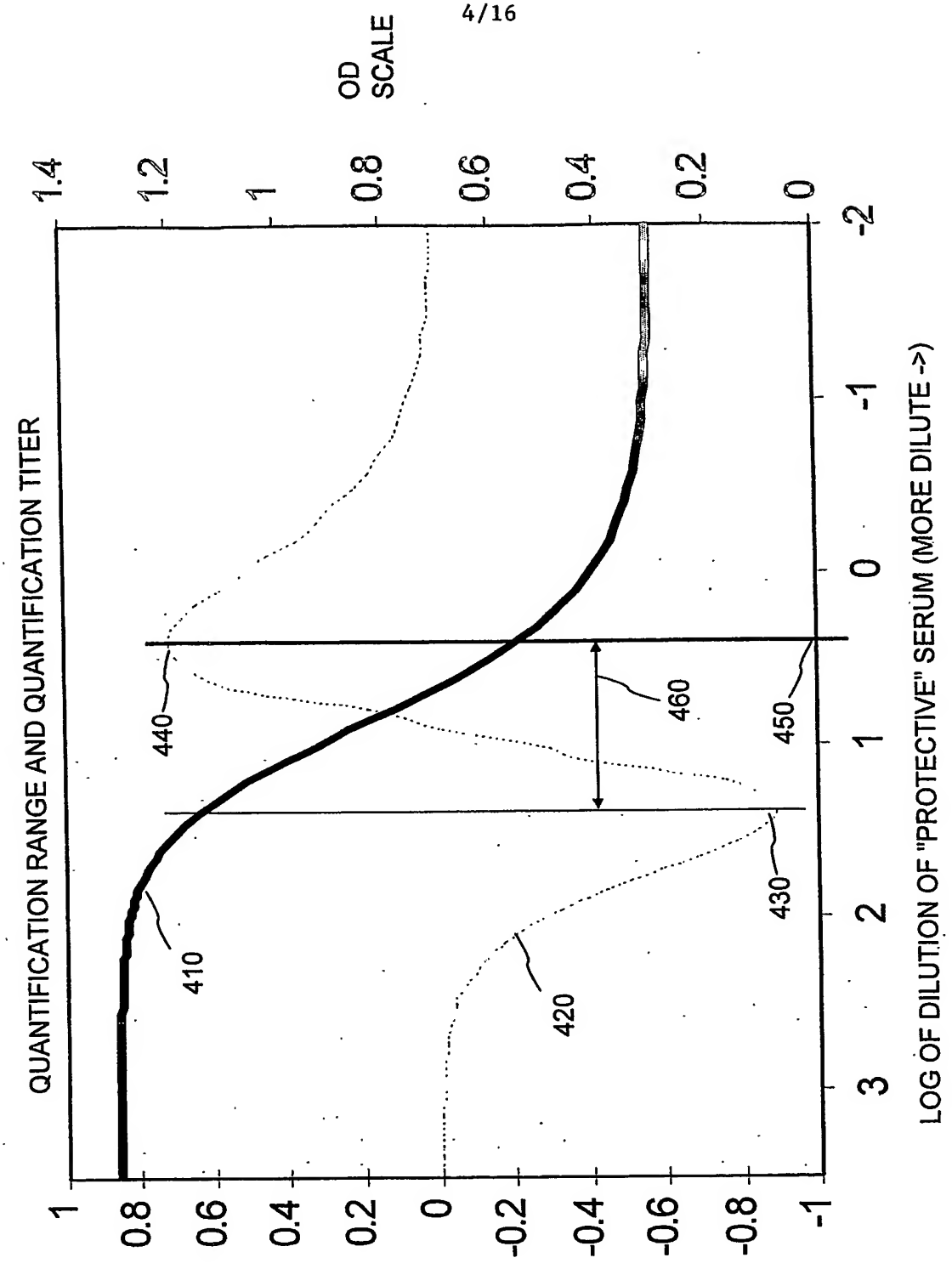

QUANTIFICATION RANGE AND QUANTIFICATION TITER
OD SCALE
SLOPE (2ND DERVIATIVE) SCALE
LOG OF DILUTION OF "PROTECTIVE" SERUM (MORE DILUTE ->)
410
420
430
440
450
460

FIG. 4

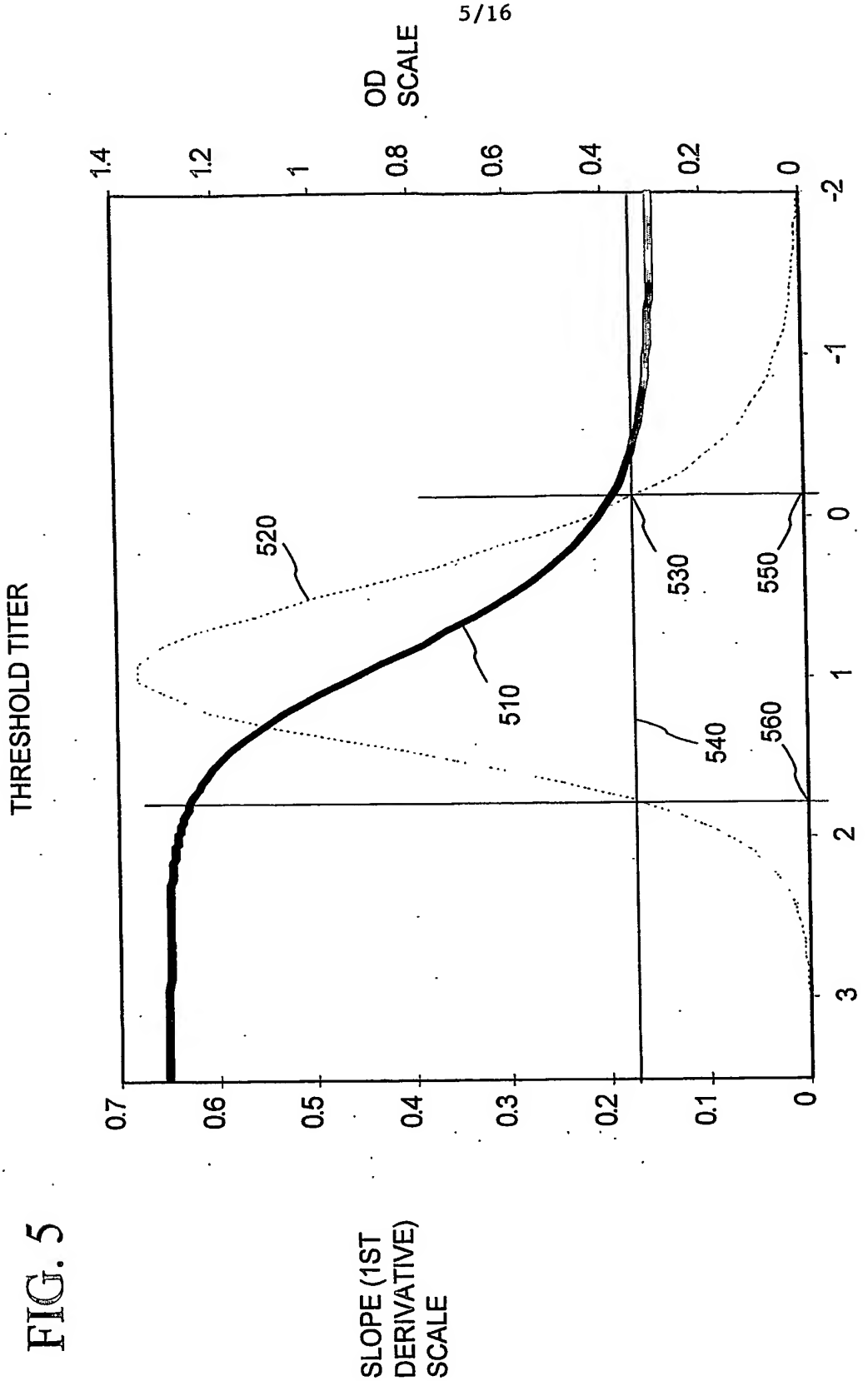

THRESHOLD TITER
OD SCALE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
520
510
530
550
540
560
-2
-1
0
1
2
3
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SLOPE (1ST DERIVATIVE) SCALE
LOG OF DILUTION OF "PROTECTIVE" SERUM (MORE DILUTE ->)

FIG. 5

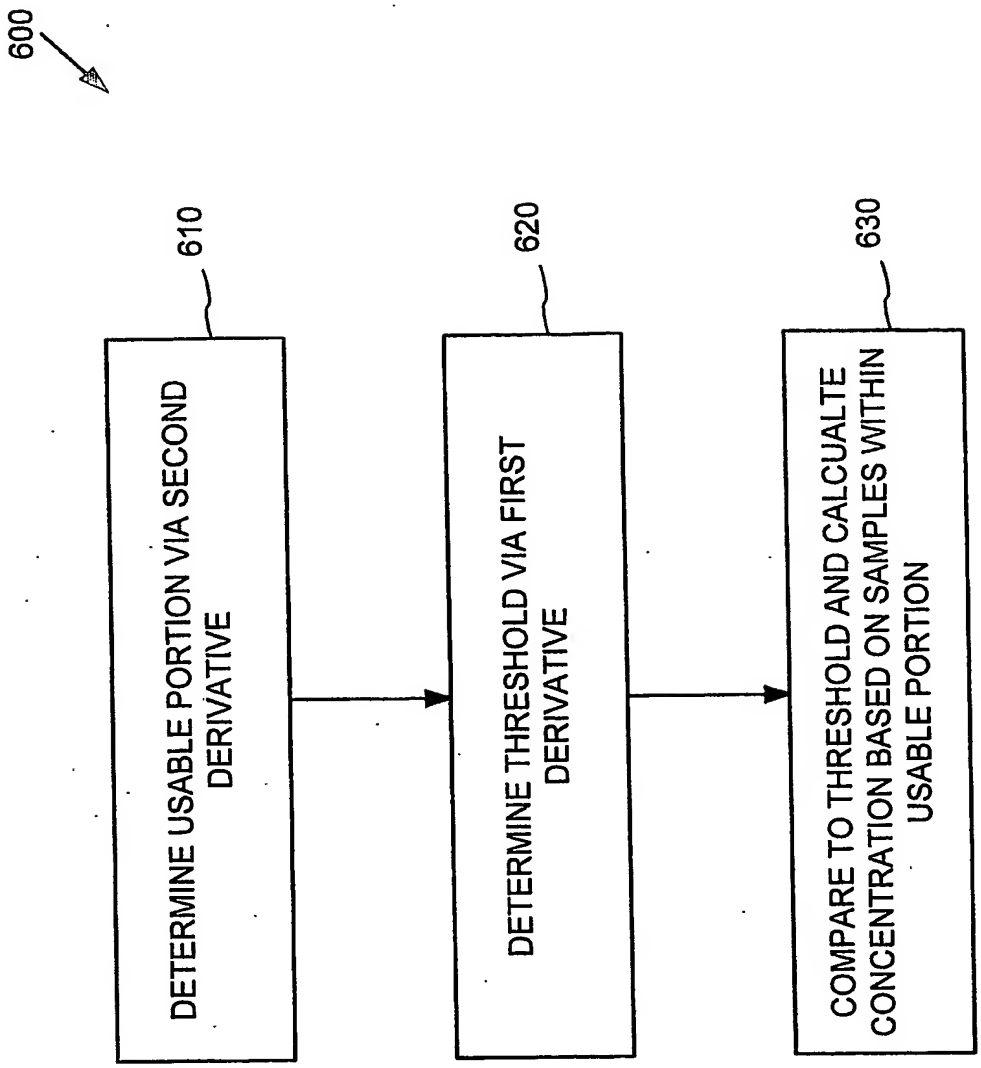

600
610
DETERMINE USABLE PORTION VIA SECOND DERIVATIVE
620
DETERMINE THRESHOLD VIA FIRST DERIVATIVE
630
COMPARE TO THRESHOLD AND CALCUALTE CONCENTRATION BASED ON SAMPLES WITHIN USABLE PORTION

FIG. 6

FIG. 7

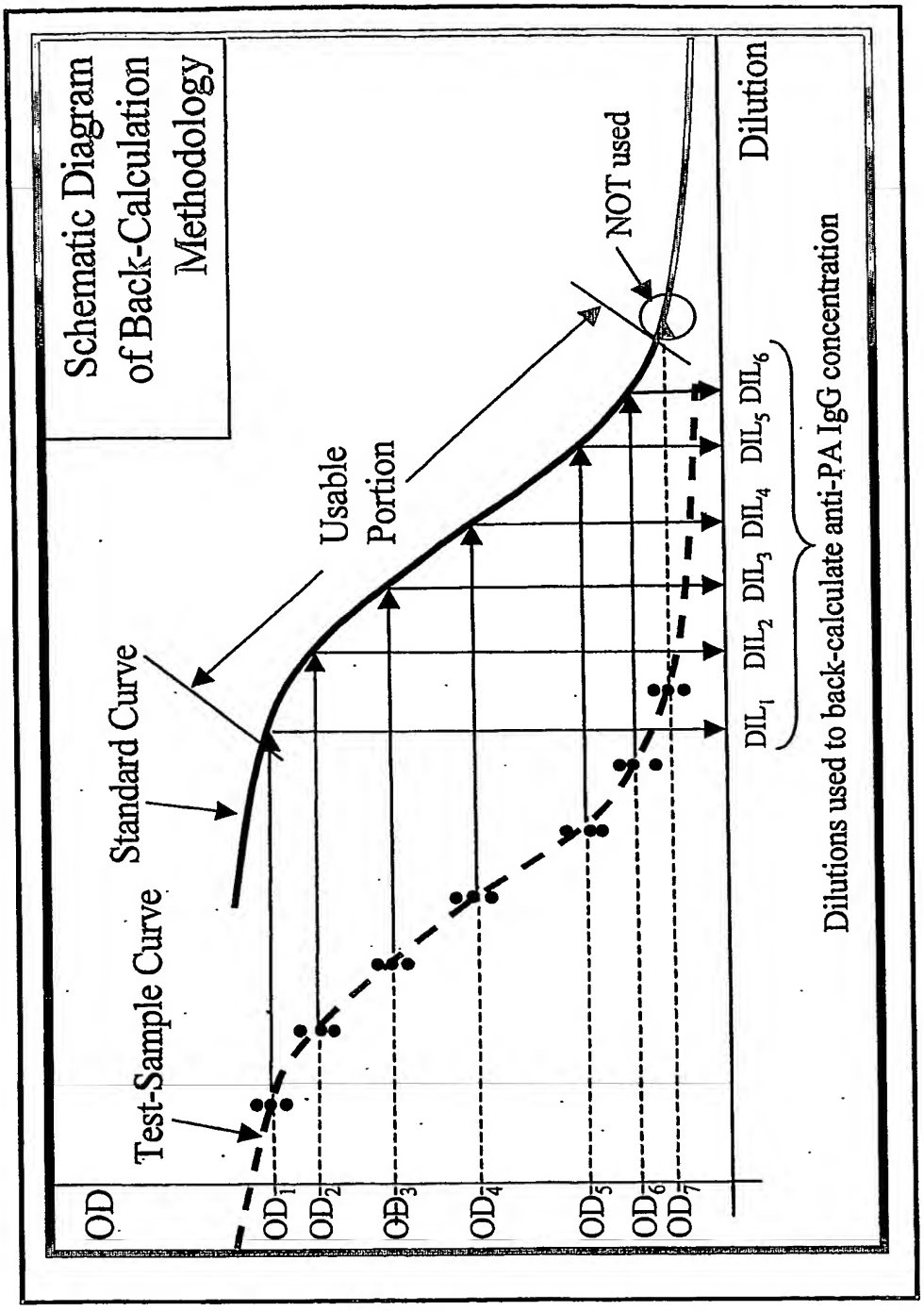

Schematic Diagram of Back-Calculation Methodology
OD
Test-Sample Curve
Standard Curve
Usable Portion
NOT used
Dilution
OD1
OD2
OD3
OD4
OD5
OD6
OD7
DIL1 DIL2 DIL3 DIL4 DIL5 DIL6
Dilutions used to back-calculate anti-PA IgG concentration

FIG. 8

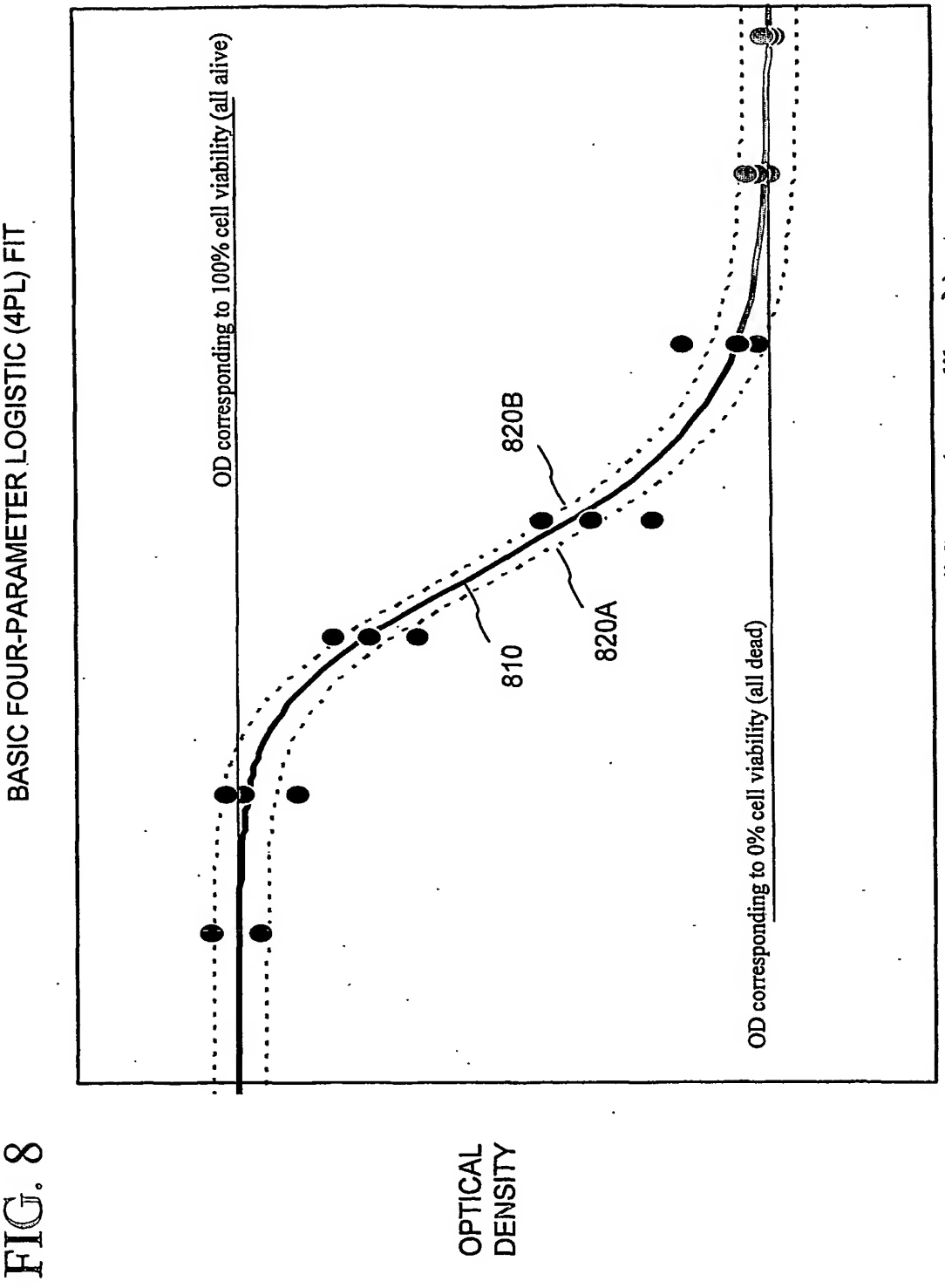
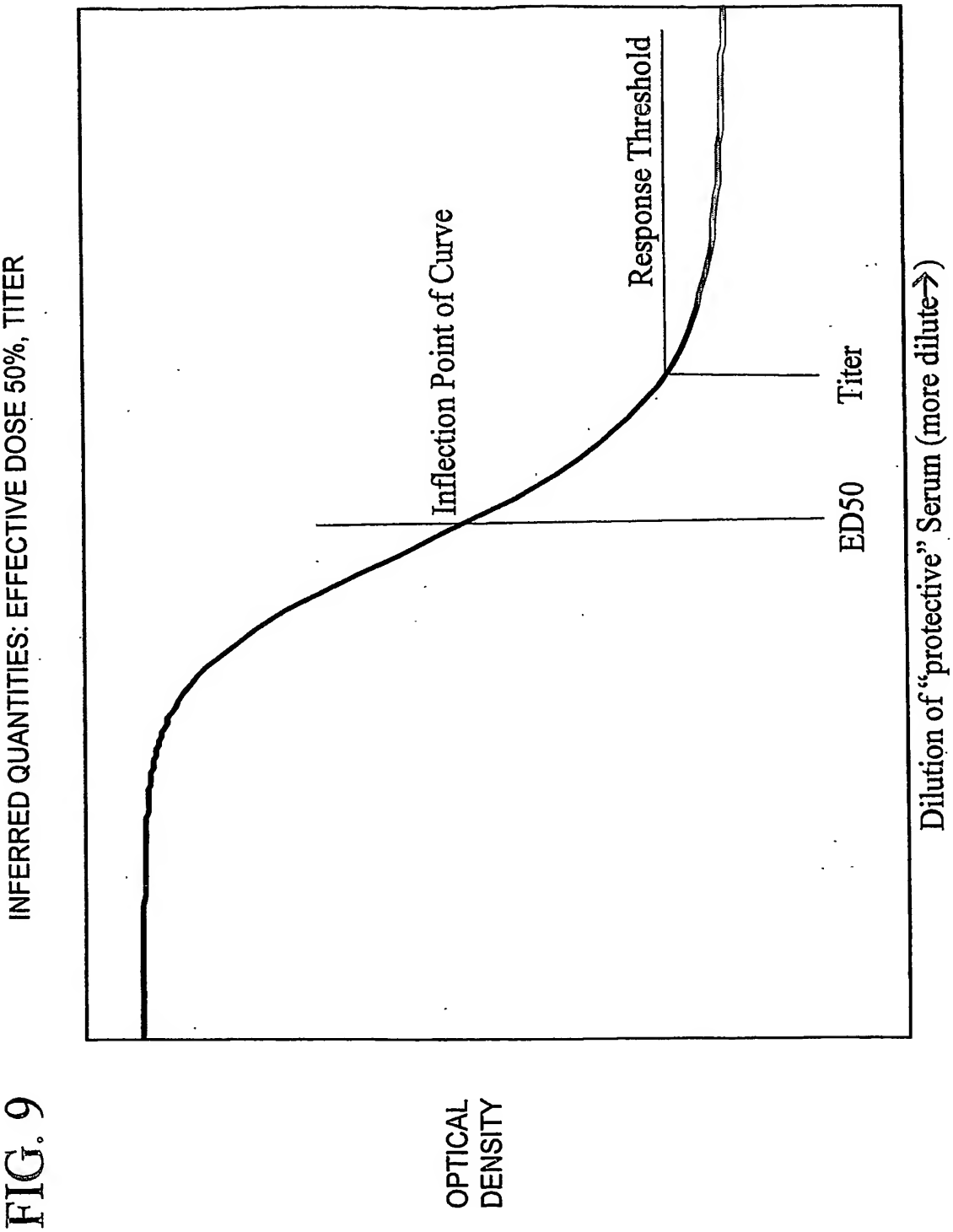

BASIC FOUR-PARAMETER LOGISTIC (4PL) FIT
OD corresponding to 100% cell viability (all alive)
820B
810
820A
OD corresponding to 0% cell viability (all dead)
OPTICAL DENSITY
Dilution of "protective" Serum (more dilute→)

FIG. 9


INFERRED QUANTITIES: EFFECTIVE DOSE 50%, TITER
OPTICAL DENSITY
Inflection Point of Curve
Response Threshold
ED50
Titer
Dilution of "protective" Serum (more dilute→)

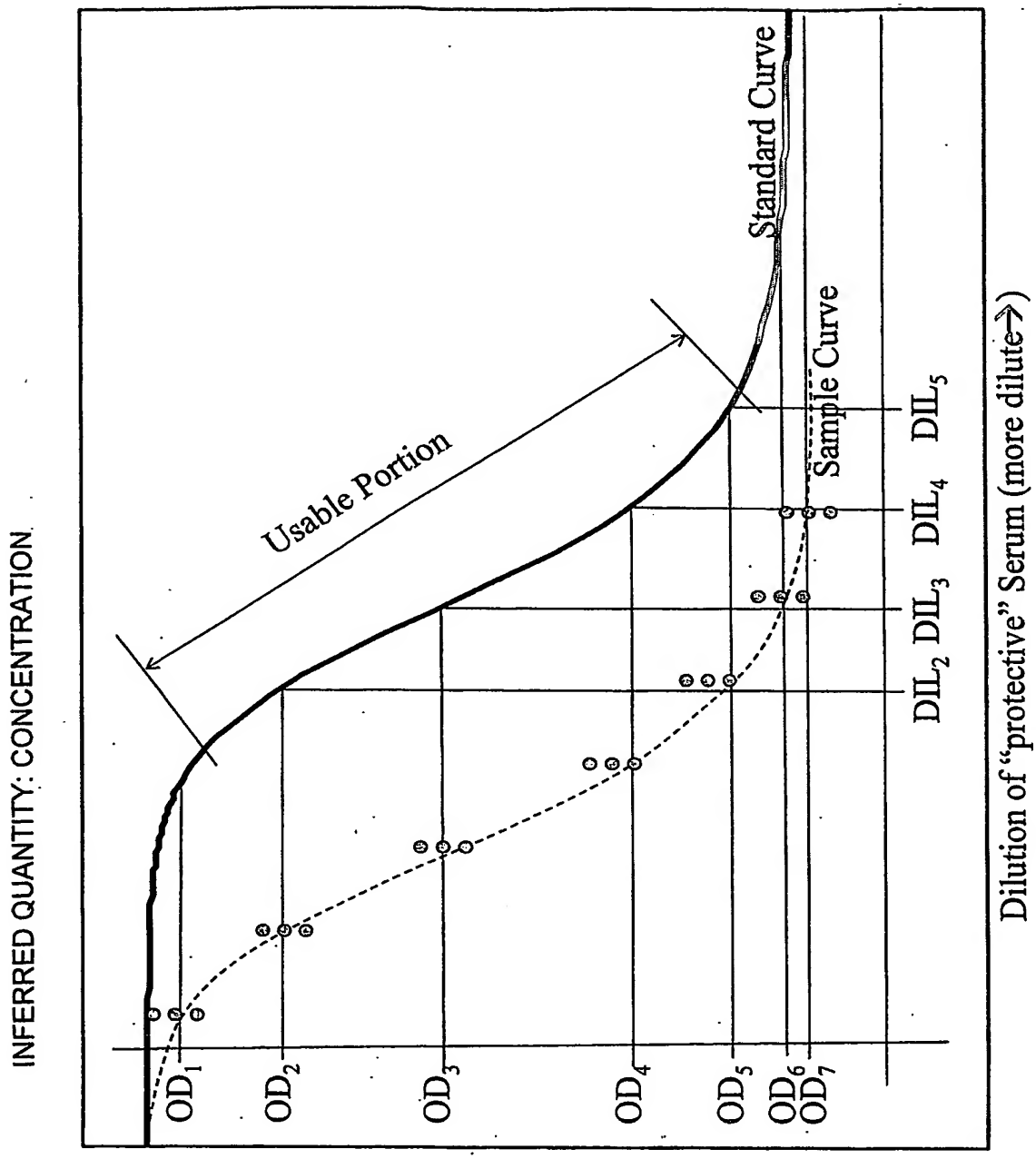

INFERRED QUANTITY: CONCENTRATION
Usable Portion
Standard Curve
Sample Curve
Dilution of "protective" Serum (more dilute→)
DIL2 DIL3 DIL4 DIL5
OD1
OD2
OD3
OD4
OD5
OD6
OD7
OPTICAL DENSITY

FIG. 10

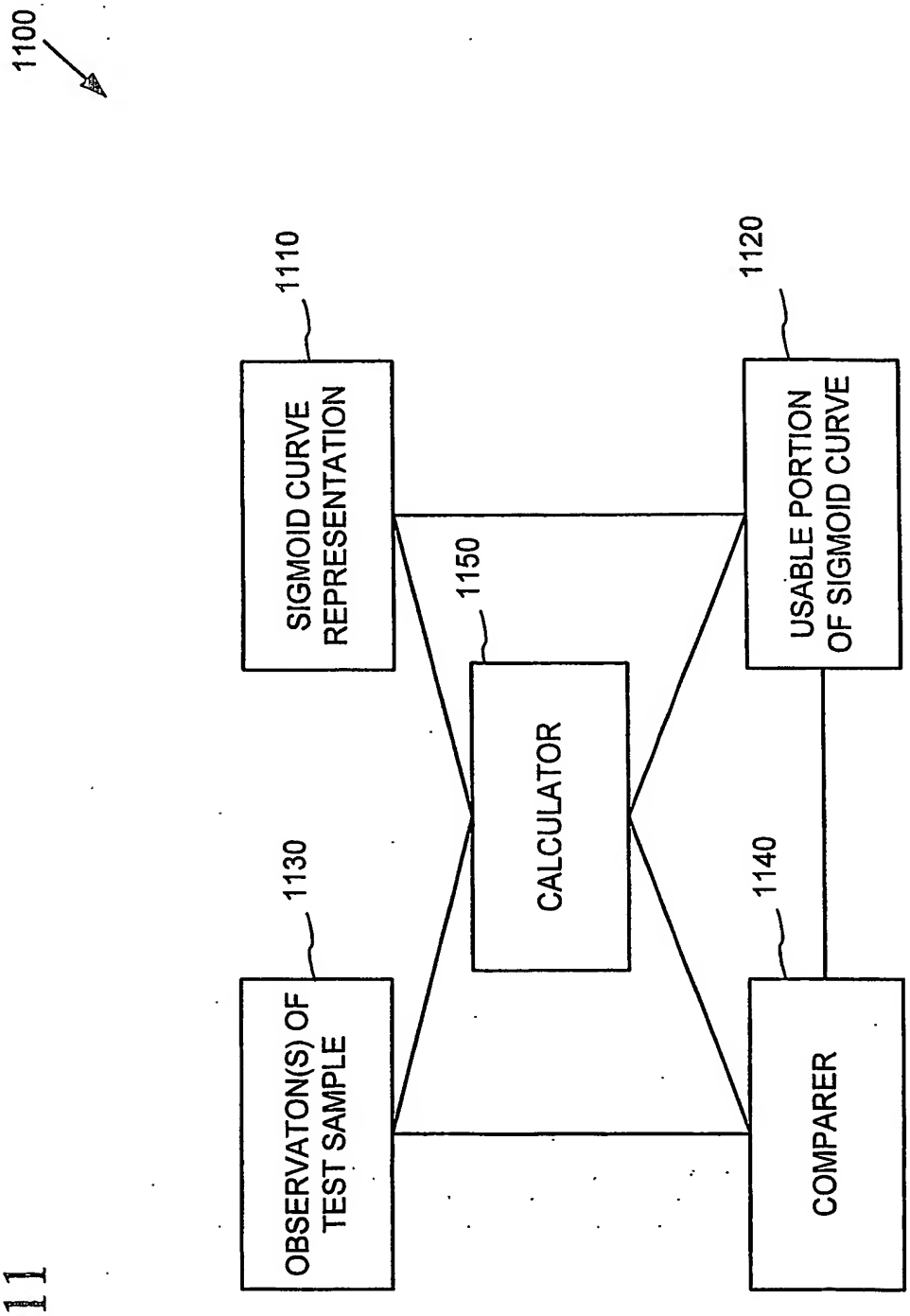

1100
1110
SIGMOID CURVE REPRESENTATION
1120
USABLE PORTION OF SIGMOID CURVE
1150
CALCULATOR
1130
OBSERVATON(S) OF TEST SAMPLE
1140
COMPARER

FIG. 11

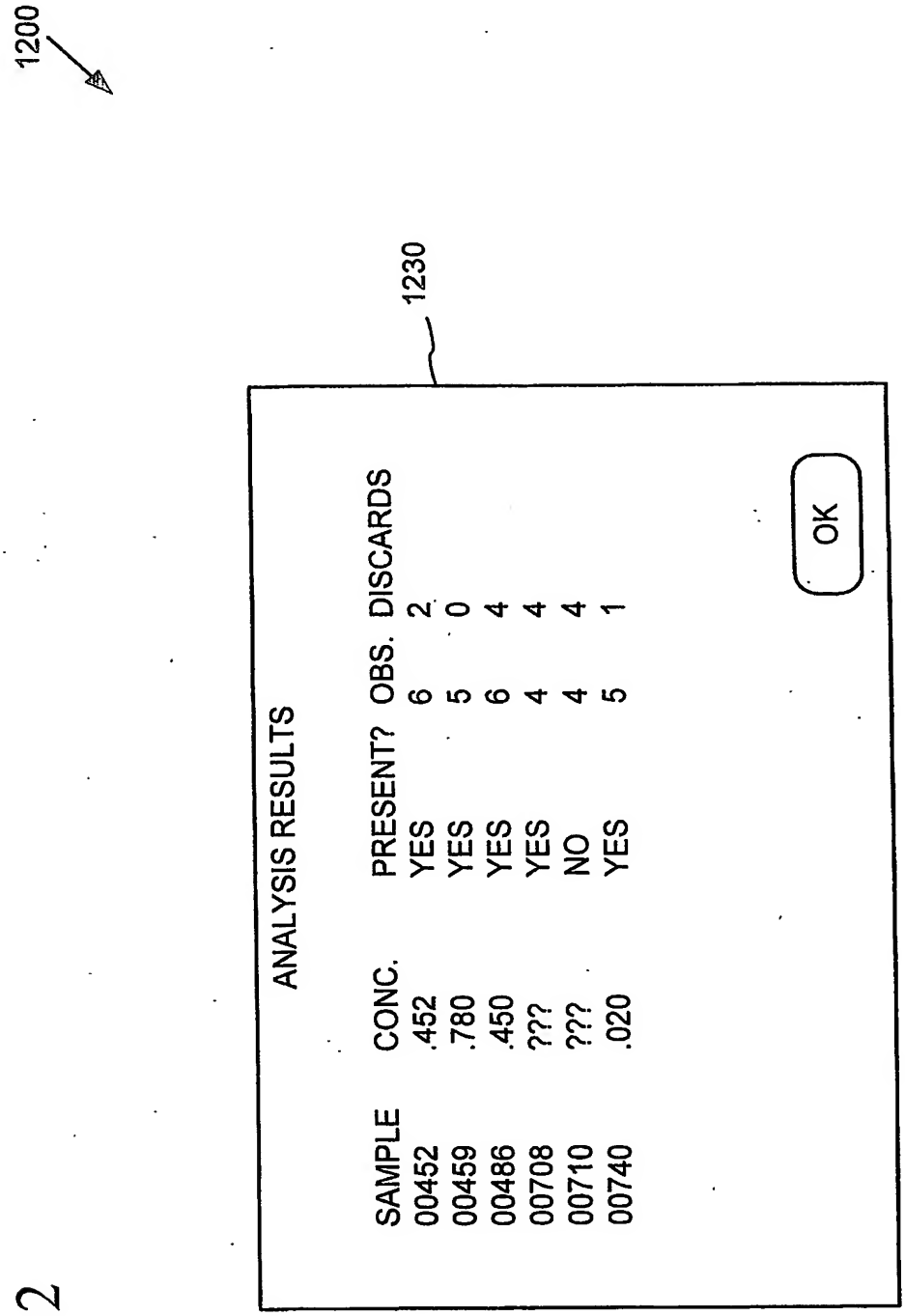

1200
1230
ANALYSIS RESULTS
SAMPLE CONC. PRESENT? OBS. DISCARDS
00452 .452 YES 6 2
00459 .780 YES 5 0
00486 .450 YES 6 4
00708 ??? YES 4 4
00710 ??? NO 4 4
00740 .020 YES 5 1
OK

FIG. 12

FIG. 13

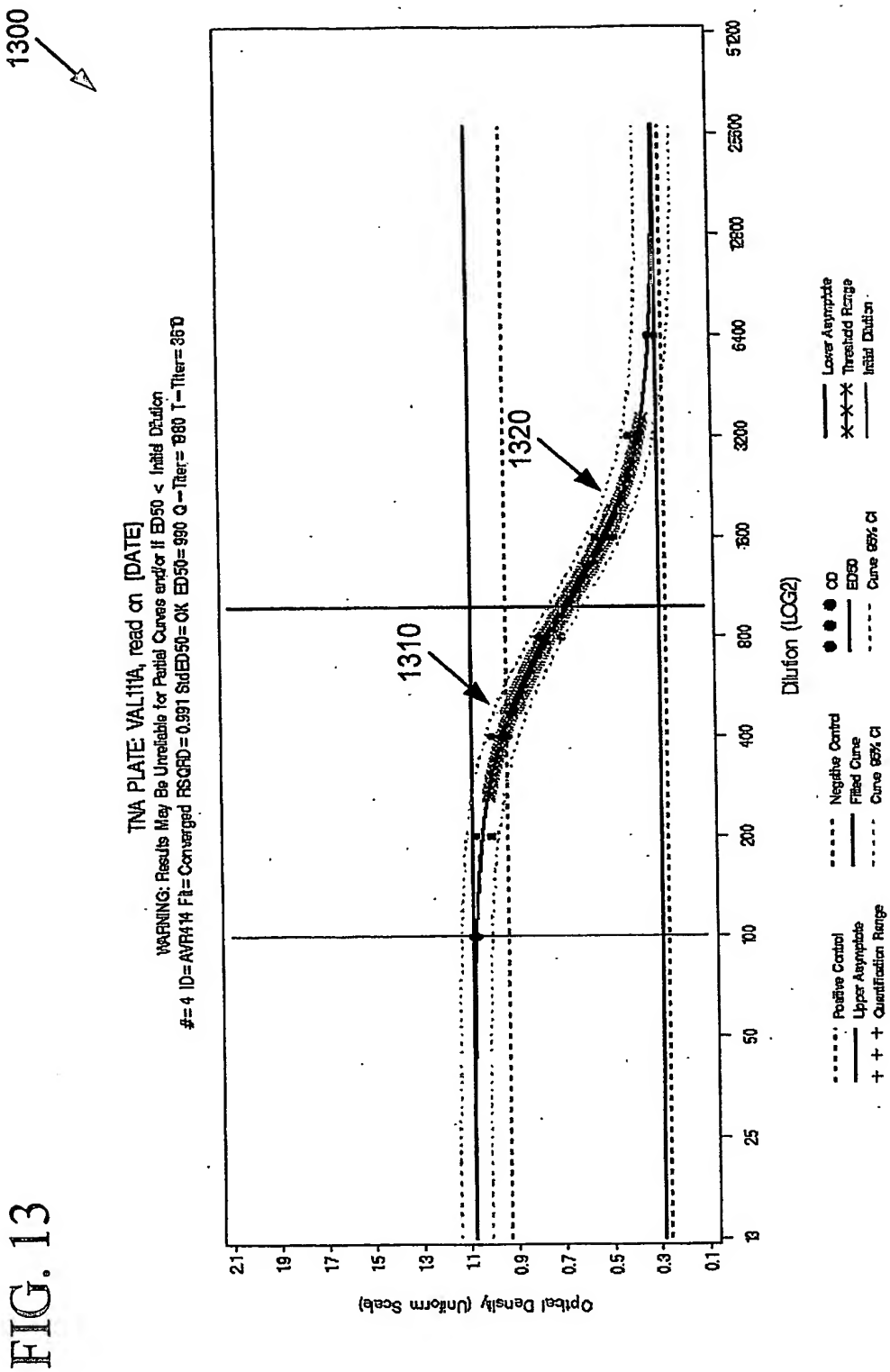

1300
TNA PLATE: VAL11A, read on [DATE]
WARNING: Results May Be Unreliable for Partial Curves and/or If ED50 < Initial Dilution
#= 4 ID=AVR414 Fit=Converged RSQRD=0.991 StdED50=OK ED50=990 Q-Titer=990 T-Titer=3610
1310
1320
Optical Density (Uniform Scale)
Dilution (LOG2)
Positive Control
Upper Asymptote
Quantification Range
Negative Control
Fitted Curve
Curve 95% CI
OD
ED50
Curve 95% CI
Lower Asymptote
Threshold Range
Initial Dilution
4PL = CONSTRAINED TO TOP OF STANDARD CURVE = METHOD: GAUSS
Negative Sample Dotted by OD diff < 0.1 and Max OD < 0.2 + Mean OD of Neg. Cntl.
Code is in ED50.ASCORE01SAS VAL11A is on QLTNA Random

FIG. 14

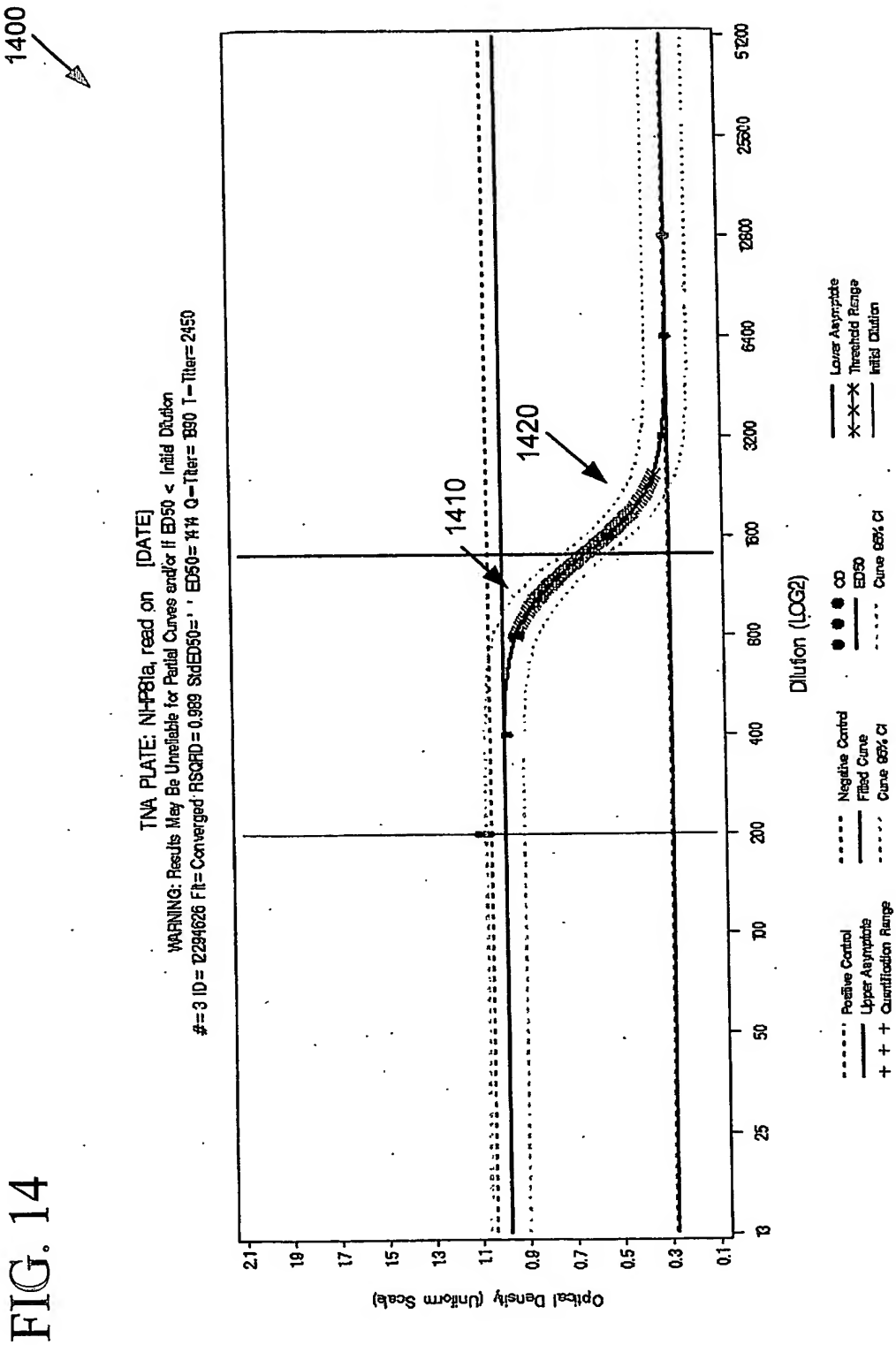

1400
1410
1420
TNA PLATE: NHP81a, read on [DATE]
WARNING: Results May Be Unreliable for Partial Curves and/or If ED50 < Initial Dilution
#=3 ID= 12294626 Fit=Converged RSQRD=0.989 StdED50=' ' ED50= 1414 Q-Titer= 1890 T-Titer=2450
Optical Density (Uniform Scale)
Dilution (LOG2)
Positive Control
Upper Asymptote
Quantification Range
Negative Control
Fitted Curve
Curve 95% CI
ED50
Curve 95% CI
Lower Asymptote
Threshold Range
Initial Dilution
4PL = CONSTRAINED TO TOP OF STANDARD CURVE = METHOD: GAUSS
1st Deriv Cutoff @ 30%
Negative Sample Deleted by OD diff < 0.1 and Max OD < 0.2 + Mean OD of Neg. Cntl.
Code is in ED50ASCORED1SAS NHP81a is on QLTNA Rawdata

FIG. 15

1500

TNA PLATE: NHP81A, READ ON [DATE] 265

WARNING: RESULTS MAY BE UNRELIABLE FOR PARTIAL CURVES AND/OR IF ED50 < INITIAL DILUTION

- SOURCE=NHP81A STD=AVR731 STD ED50=1211 COMPUTATION METHOD=GAUSS ANALYST=HL READDATE=[DATE]
--

SAMNO	SAMPLE	CURVE FIT STATUS	INITIAL DILUTION	FLAGNADA	FLAG34	FLAG ED50 OF STD	RSQRD	FLAGRSQRD
1	12275434	CONVERGED	200				0.985	
2	12292526	CONVERGED	100				0.982	
3	12294626	CONVERGED	200				0.989	
4	AVR731	CONVERGED	200		OK	??	0.997	

SAMNO	ED50	WARNING: LOW TITER	QUANTIF. TITER	THRESHOLD TITER	NF50	OD RANGE OF STD	MEAN OD OF POS. CNTRL.	STD DEV OD OF POS. CNTRL.
1	1299		1740	2220	1.07	.	1.01	0.037
2	870		1180	1560	0.72	.	1.14	0.095
3	1414		1890	2450	1.17	.	1.04	0.029
4	1211		1810	2550	1.00	0.73	0.94	0.015

FIG. 16

1600

SAMNO	CV OF OD OF POS. CNTRL.	DELTA: BOT. OD TRIPLICATE & NEG. CNTRL.	MEAN OD OF NEG. CNTRL.	STD DEV OD OF NEG. CNTRL.	CV OF OD OF NEG. CNTRL.	UPPER ASYMP.	LOWER ASYMP.	MAXIMUM OD TRIPLE
1	4%	0.00	0.28	0.002	1%	0.98	0.29	1.08
2	8%	0.01	0.28	0.002	1%	0.98	0.28	1.09
3	3%	0.01	0.28	0.002	1%	0.98	0.28	1.07
4	2%	0.01	0.28	0.002	1%	0.98	0.27	0.99

SAMNO	LOWEST OD TRIPLE	MAXIMUM CV OF ODS	POINTS-BASED IGG CONC.	CURVE-BASED IGG CONC.	VERSION	QCCHECK
1	0.28	4%	260.3	190.6	ED50.45CORE01	
2	0.27	6%	136.7	126.6	ED50.45CORE01	
3	0.27	3%	233.6	204.8	ED50.45CORE01	
4	0.27	3%	177.3	171.9	ED50.45CORE01	??